

Binding of C-reactive protein to human neutrophils

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The binding of radiolabelled ^{125}I -CRP to human neutrophils has been characterised according to pH, temperature and time dependence. The binding of ^{125}I -CRP was saturable, very fast (<2 min at 22°C), and the labelled protein was displaced by unlabelled CRP and aggregated human IgG. The dissociation constant was 3.2×10^{-8} M at pH 7.4, 22°C and 8.8×10^{-8} M at pH 6.0, 22°C . The calculated number of binding sites was $5 - 20 \times 10^4$ per cell at pH 7.4, 22°C . An association with an F_c -type receptor is suggested, since aggregated IgG was able to displace specifically CRP.

C-reactive protein; Neutrophil; Receptor

1. INTRODUCTION

Elevated amounts of C-reactive protein are found in human serum during the acute-phase response, as a result of inflammation and tissue injury [1]. Levels of CRP can increase from a median level of less than $1 \mu\text{g/ml}$ by up to 1000-fold in less than 48 h as a result of enhanced biosynthesis in liver hepatocytes which is mediated by a macrophage-derived factor suggested to be interleukin-1 [1,2]. CRP is characterized by its binding in a Ca^{2+} -dependent manner to C-polysaccharide [3] and in particular to the ligand phosphocholine [4].

CRP has been found at sites of tissue lesion [5,6]. Neutrophilia and neutrophil migration into sites of inflammation and tissue injury is a com-

mon immune defense response [7]. Thus, studying the binding of CRP to neutrophils and its possible consequence on metabolic and functional activity of the cells would be of interest. Reports have indicated varying effects of CRP on neutrophil function such as motility [8], secretion of enzymes [6] and phagocytosis of certain bacteria [9]. In the presence of the stimulant PMA, enhancement of phagocytosis of CRP and PC coated sheep erythrocytes by neutrophils suggested the possibility of a specific cellular receptor for CRP [10]. We have previously studied the effect of CRP on neutrophil functions, e.g. superoxide production, secretion, chemotaxis and phagocytosis [11], which also suggested that CRP binds to a specific receptor.

We have characterised several parameters of the interaction of CRP with human neutrophils at pH 7.4 including saturation and competition binding, kinetics and temperature dependence with purified radiolabelled ^{125}I -CRP. Since at sites of inflammation the pH is found to be 6–7 [12], binding studies were also performed at pH 6.0. The possibility that CRP may compete with IgG for a F_c type receptor was also investigated.

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Abbreviations: CRP, C-reactive protein; PMA, phorbol 12-myristate 13-acetate; Con A, concanavalin A; PC, phosphocholine

2. MATERIALS AND METHODS

2.1. Purification of human C-reactive protein

CRP was purified from pooled human sera using an affinity column of phosphoryl-ethanolamine-agarose (Sigma, St. Louis, MO) [13], followed by passage on a Sephadex G-200 (Pharmacia, Uppsala) column [14].

2.2. Iodination of CRP

CRP was iodinated by the iodogen method '15' with carrier-free Na^{125}I , and an Iodobead (Pierce, Rockwell, IL), and separated from the free iodine on a column of Sephadex G-25 (Pharmacia). The specific activity of various preparations was 2–7 $\mu\text{Ci}/\mu\text{g}$.

2.3. Neutrophils

Neutrophils were isolated from human blood (Blood Bank, Jaffa, Israel) as described [16]. Preparations contained at least 95% neutrophils of at least 98% viability as found by exclusion of trypan blue or eosin Y.

2.4. Binding assay

Binding studies were performed at 4, 22 and 37°C for 30, 20 and 15 min, respectively, at a final volume of 250 μl PBS, pH 7.4 or pH 6.0. Cells were incubated with gentle agitation in plastic tubes (Nunc, 70 \times 12 mm, $3\text{--}5 \times 10^6$ neutrophils/tube) with ^{125}I -CRP ($\sim 0.05 \mu\text{g}$) together with various concentrations of unlabelled CRP. Incubations were terminated by rapidly

diluting with 1.5 ml cold PBS, followed by centrifugation at $300 \times g$ (Beckman TJ-6) for 7 min at 4°C. The supernatant was discarded by aspiration and the pellet washed twice more with cold PBS. Non-specific binding was defined as the amount of binding not inhibited by 50 $\mu\text{g}/\text{ml}$ of unlabelled CRP and was usually equal to about 40–50% of the total counts bound (22°C, pH 7.4). Specific binding was defined as the total amount of ^{125}I -CRP bound minus the non-specific binding. The binding referred to is that of specific binding. The ^{125}I -CRP was determined by counting in a Kontron MD-480 gamma counter, and all results are the means of triplicate determinations.

3. RESULTS

The specific saturation binding of ^{125}I -CRP at pH 7.4, and at 4, 22 and 37°C is shown in fig.1. Displacement of ^{125}I -CRP by increasing concentrations of CRP is shown in fig.2A. Scatchard analysis of the binding gave a dissociation constant of 3.2×10^{-8} M, at pH 7.4, 22°C, as determined from the Scatchard plot (fig.2A, inset). The number of binding sites per cell was $5\text{--}20 \times 10^4$.

Displacement of ^{125}I -CRP by CRP in the presence of 10 $\mu\text{g}/\text{ml}$ heat-aggregated human IgG is shown in fig.2B. The displacement of ^{125}I -CRP by increasing concentrations of heat-aggregated human IgG is shown in fig.2B (inset).

Kinetic studies at 37 and 22°C reveal very fast binding of ^{125}I -CRP, with complete saturation within 2 min, while at 4°C this occurs within 5–10 min (fig.3). After reaching saturation, there is a decrease in the specific binding of CRP to about 70–90% of the maximal specific binding found. At pH 6.0, the degree of non-specific binding is reduced to less than 10% of the total binding. Saturation binding is observed at 4, 22 and 37°C as shown in fig.4. Maximal displacement of ^{125}I -CRP by CRP at 22°C occurred at a higher concentration of CRP (fig.5). Scatchard analysis gave a dissociation constant of 8.8×10^{-8} M (fig.5, inset).

No significant change in the binding of ^{125}I -CRP to neutrophils was observed when the cells were initially stimulated with either PMA or Con A. The presence of PC (CRP:PC, 1:5) resulted in a small increase in the affinity of CRP binding, with a dissociation constant of 2.9×10^{-8} M. PC alone at

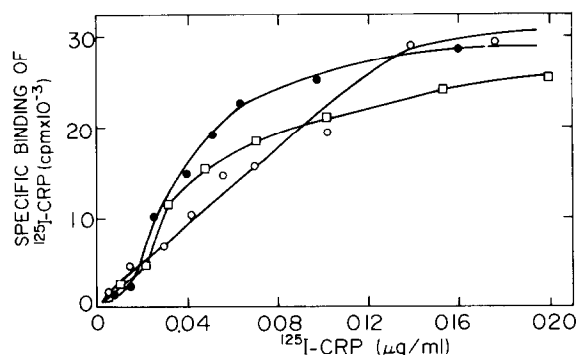


Fig.1. Specific binding of ^{125}I -CRP to human neutrophils at pH 7.4. 4°C (○—○), 22°C (●—●), 37°C (□—□).

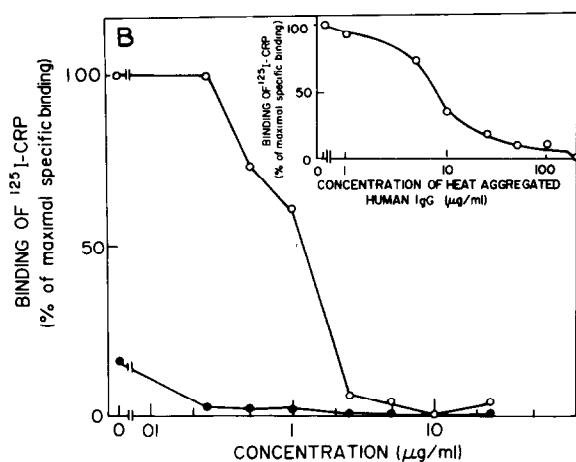
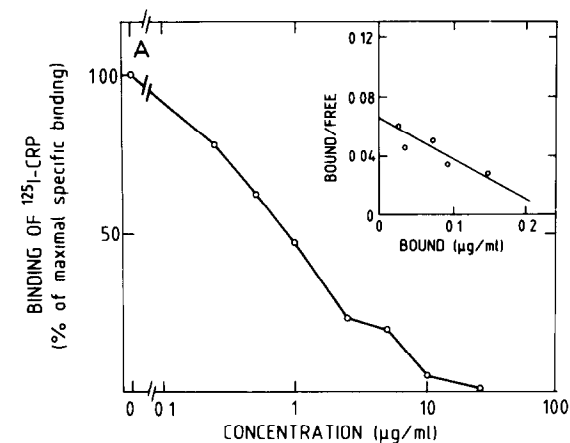


Fig. 2. (A) Displacement of ^{125}I -CRP by CRP from neutrophils, pH 7.4, 22°C . Concentration of ^{125}I -CRP was 0.2–0.4 $\mu\text{g/ml}$. (Inset) Scatchard plot. (B) Displacement of ^{125}I -CRP by CRP in the absence (○—○) or presence (●—●) of 10 $\mu\text{g/ml}$ heat-aggregated IgG from neutrophils, pH 7.4, 22°C . (Inset) Displacement of ^{125}I -CRP by various concentrations of heat-aggregated IgG.

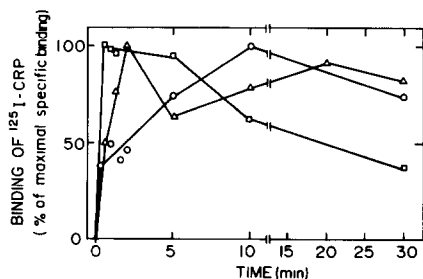


Fig. 3. Time course of specific binding of ^{125}I -CRP to neutrophils at 4°C (○—○), 22°C (□—□) and 37°C (Δ—Δ). Concentration of ^{125}I -CRP was 0.28 $\mu\text{g/ml}$.

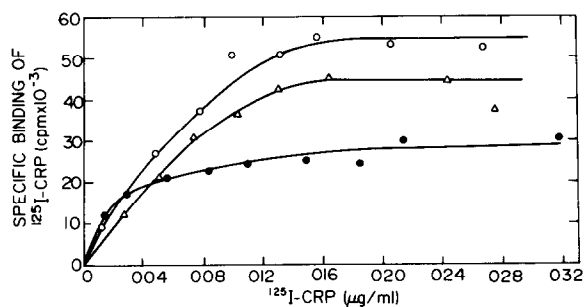


Fig. 4. Specific binding of ^{125}I -CRP at pH 6.0 to neutrophils at 4°C (●—●), 22°C (○—○) and 37°C (Δ—Δ).

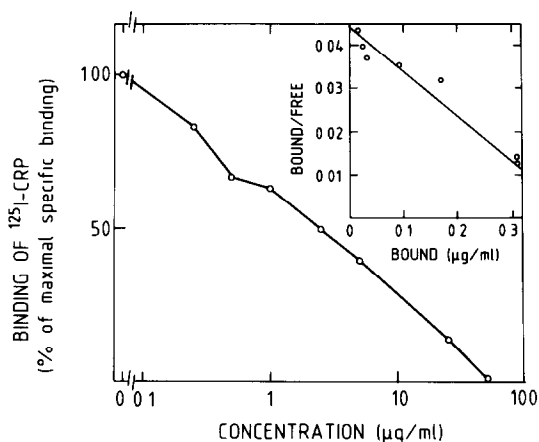


Fig. 5. Displacement of ^{125}I -CRP by CRP from neutrophils at pH 6.0, 22°C . (Inset) Scatchard plot.

1000-times the concentration of ^{125}I -CRP displaced less than 5% of specifically bound CRP (not shown).

4. DISCUSSION

The present results point to the existence of specific receptors for CRP on the human neutrophil cell surface. As a consequence of the specific association of CRP with these loci, a series of cellular functions is manifested [11].

Based on the competition binding experiments with heat-aggregated IgG, it is suggested that the CRP receptors are related to the IgG receptors. Previous studies, though of not direct binding, have indicated association of CRP with F_c -type receptors in other cells. In vitro studies with monocytes show that following complement fixa-

tion by CRP binding to a particulate ligand, the presence of both CRP and the fixed complement is required for the internalization of the immune complex by the monocytes [17]. Similarly, in vitro experiments with mouse macrophages suggest that since phagocytosis of CRP bound to sheep red blood cells coated with antibody to surface antigen was inhibited by IgG, cell interaction occurs via the F_c -type receptor [18].

On completion of the present work, the binding of CRP to human neutrophils was also reported [19]. A dissociation constant of 10×10^{-8} M and $1-3 \times 10^6$ binding sites per cell at 37°C were found. Binding was detected, however, only in the presence of an additional factor found in heat-inactivated serum [19]. On the other hand, in our experiments no such factor was required for binding. Moreover, we find that saturable binding occurs at a CRP concentration of $< 5 \mu\text{g/ml}$, pH 7.4, rather than $\sim 30 \mu\text{g/ml}$. The former concentration is within the range at which CRP exerts maximal enhancement several functions of stimulated neutrophils, including superoxide production, secretion, chemotaxis and phagocytosis. At higher CRP concentrations ($> 20 \mu\text{g/ml}$), an inhibition of neutrophil function was observed [11].

The results indicate that CRP binds to a specific receptor on the neutrophil surface, and which appears to bear a relationship to the F_c receptor. The occurrence of specific receptors for CRP on surfaces of phagocytes and the consequent effect of protein-cell binding upon various cellular functions strongly support the notion that CRP is intimately involved in defense mechanisms which are associated with inflammation. Further work on the characterization of the CRP receptor using isolated neutrophil membranes is in progress.

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